

Supplementary Tables

Table 1. Single stranded oligonucleotides used in this study (listed 5' to 3').

Strand Name	Sequence
pp-luc-ss	CGUACGCGGAAUACUUCGAUA
pp-luc-ss-4SU(20)	CGUACGCGGAAUACUUCGA <u>UA</u>
pp-luc-as	UCGAAGUAUUCCGCGUACGUG
pp-luc-as-4SU(20)	UCGAAGUAUUCCGCGUACG <u>UG</u>
pp-luc-ss-A(1)	<u>A</u> GUACGCGGAAUACUUCGAUA
pp-luc-ss-C(19)	CGUACGCGGAAUACUUC <u>CG</u> UA
sod1-as	GUCACAUUGCCCAAGUCU UU
sod1-as-4SU(20)	GUCACAUUGCCCAAGUCU <u>CU</u> UU
sod1-ss	GAGACUUGGGCAAUGUGACUU
sod1-ss-4SU(20)	GAGACUUGGGCAAUGUGAC <u>UU</u>
sod1-as-U(1)	<u>U</u> AGACUUGGGCAAUGUGACUU
sod1-as-A(19)	GAGACUUGGGCAAUGUGA <u>AU</u>
EGFP-as	UGC GC CU CC U GG AC GU AG CC UU
EGFP-as-4SU(20)	UGC GC CU CC U GG AC GU AG CC <u>UU</u>
EGFP-ss	GGC U AC GU CC A G G C G C A U U
EGFP-ss-4SU(20)	GGC U AC GU CC A G G C G C <u>UU</u>
EGFP-ss-U(1)	<u>U</u> GGC U AC GU CC A G G C G C A U U
EGFP-ss-C(19)	GGC U AC GU CC A G G C G C <u>UU</u>
EGFP-as-5'OCH3	mTGCGCUCCUGGACGUAGCCUU
EGFP-ss-5'OCH3	mTGC U C A G U C C A G G A G C G C A U U

U denotes 4-thiouracil; mT denotes 5'-O-methyl thymidine; underline denotes nucleotide that is mismatched to its complementary sequence.

Table 2. Nucleic acid duplexes targeting EGFP used in Fig. 2 (top strand listed 5' to 3', bottom strand listed 3' to 5').

Strand Name	Sequence
RNAss	p*–GGCUACGUCCAGGAGCGCAUU
RNAas	UUCCGAUGCAGGUCCUCGCGU–p*
DNAss	p*–GGCTACGTCCAGGAGCGCATT
DNAas	TTCCGATGCAGGTCCCTCGCGT–p*
RNAss	p*–GGCUACGUCCAGGAGCGCAUU
DNAas	TTCCGATGCAGGTCCCTCGCGT–p*
DNAss	p*–GGCTACGTCCAGGAGCGCATT
RNAas	UUCCGAUGCAGGUCCUCGCGU–p*

* denotes 5'-phosphorylation with γ -³²P.

Table 3. Nucleic acid duplexes targeting pp-luciferase (pp-luc) (top strand listed 5' to 3', bottom strand listed 3' to 5').

Duplex Name	Strand Name	Sequence
a	pp-luc-as	p*-UCGAAGUAUCCCGGUACGUG
	pp-luc-ss	AUAGCUUCAUAAGGCGCAUGC-p*
b	pp-luc-as-4SU(20)	p*-UCGAAGUAUCCCGGUACG UG
	pp-luc-ss	AUAGCUUCAUAAGGCGCAUGC-p
c	pp-luc-as-4SU(20)	p -UCGAAGUAUCCCGGUACG UG
	pp-luc-ss	AUAGCUUCAUAAGGCGCAUGC-p*
d	pp-luc-as	p -UCGAAGUAUCCCGGUACGUG
	pp-luc-ss-4SU(20)	A UAGCUUCAUAAGGCGCAUGC-p*
e	pp-luc-as	p*-UCGAAGUAUCCCGGUACGUG
	pp-luc-ss-4SU(20)	A UAGCUUCAUAAGGCGCAUGC-p
f	pp-luc-as-4SU(20)	p*-UCGAAGUAUCCCGGUACG UG
	pp-luc-ss-4SU(20)	A UAGCUUCAUAAGGCGCAUGC-p*
g	pp-luc-as-4SU(20)	p*-UCGAAGUAUCCCGGUACG UG
	pp-luc-ss-A(1)	AUAGCUUCAUAAGGCGCAUG <u>A</u> -P
h	pp-luc-as-4SU(20)	p*-UCGAAGUAUCCCGGUACG UG
	pp-luc-ss-C(19)	<u>A</u> UCGUUCAUAAGGCGCAUGC-P
i	pp-luc-as-4SU(20)	p*-UCGAAGUAUCCCGGUACG UG
	<i>pp-luc-ss</i>	ATAGCTTCATAAGGCGCATGC-p
j	<i>pp-luc-as</i>	p -TCGAAGTATTCCCGTACGTG
	pp-luc-ss-4SU(20)	A UAGCUUCAUAAGGCGCAUGC-p*

U denotes 4-thiouracil; underline denotes nucleotide that is mismatched to its complementary sequence; italics denote DNA strands; p denotes 5'-phosphorylation; * denotes 5'-phosphorylation with $\gamma^{32}\text{P}$.

Table 4. Nucleic acid duplexes targeting human Cu, Zn-Superoxide dismutase (sod1) (top strand listed 5' to 3', bottom strand listed 3' to 5').

Duplex Name	Strand Name	Sequence
a	sod1-ss	p* -GAGACUUGGGCAAUGUGACUU
	sod1-as	UUCUCUGAAC <u>CCGUUACACUG-P*</u>
b	sod1-ss-4SU(20)	p* -GAGACUUGGGCAAUGUGAC UU
	sod1-as	UUCUCUGAAC <u>CCGUUACACUG-P</u>
c	sod1-ss-4SU(20)	p -GAGACUUGGGCAAUGUGAC UU
	sod1-as	UUCUCUGAAC <u>CCGUUACACUG-P*</u>
d	sod1-ss	p -GAGACUUGGGCAAUGUGACUU
	sod1-as-4SU(20)	UU CUCUGAAC <u>CCGUUACACUG-P*</u>
e	sod1-ss	p* -GAGACUUGGGCAAUGUGACUU
	sod1-as-4SU(20)	UU CUCUGAAC <u>CCGUUACACUG-P*</u>
f	sod1-ss-4SU(20)	p* -GAGACUUGGGCAAUGUGAC UU
	sod1-as-4SU(20)	UU CUCUGAAC <u>CCGUUACACUG-P*</u>
g	sod1-ss-4SU(20)	p* -GAGACUUGGGCAAUGUGAC UU
	sod1-as-U(1)	UUCUCUGAAC <u>CCGUUACACUU-P</u>
h	sod1-ss-4SU(20)	p* -GAGACUUGGGCAAUGUGAC UU
	sod1-as-A(19)	<u>UU</u> AUCUGAAC <u>CCGUUACACUG-P</u>
i	sod1-ss-4SU(20)	p* -GAGACUUGGGCAAUGUGAC UU
	sod1-as	<i>UUCUCUGAAC<u>CCGUUACACUG-P</u></i>
j	sod1-ss	p -GAGACUUGGGCAAUGUGACUU
	sod1-as-4SU(20)	UU CUCUGAAC <u>CCGUUACACUG-P*</u>

U denotes 4-thiouracil; underline denotes nucleotide that is mismatched to its complementary sequence; p denotes 5'-phosphorylation; * denotes 5'-phosphorylation with $\gamma^{32}\text{P}$.

Table 5. Nucleic acid duplexes targeting EGFP (top strand listed 5' to 3', bottom strand listed 3' to 5').

Duplex Name	Strand Name	Sequence
a	EGFP-as	p*-UGC ^U GUAGCCUU
	EGFP-ss	UUACGCGAGGACCUGCAUCGG-p*
b	EGFP-as-4SU(20)	p*-UGC ^G GUAGCCUU
	EGFP-ss	UUACGCGAGGACCUGCAUCGG-p
c	EGFP-as-4SU(20)	p*-UGC ^G GUAGCCUU
	EGFP-ss	UUACGCGAGGACCUGCAUCGG-p*
d	EGFP-as	p*-UGC ^G GUAGCCUU
	EGFP-ss-4SU(20)	UUACGCGAGGACCUGCAUCGG-p*
e	EGFP-as	p*-UGC ^G GUAGCCUU
	EGFP-ss-4SU(20)	UUACGCGAGGACCUGCAUCGG-p
f	EGFP-as-4SU(20)	p*-UGC ^G GUAGCCUU
	EGFP-ss-4SU(20)	UUACGCGAGGACCUGCAUCGG-p*
g	EGFP-as-4SU(20)	p*-UGC ^G GUAGCCUU
	EGFP-ss-U(1)	UUACGCGAGGACCUGCAUCGU-p
h	EGFP-as-4SU(20)	p*-UGC ^G GUAGCCUU
	EGFP-ss-C(19)	UU <u>C</u> CGCGAGGACCUGCAUCGG-p
k	EGFP-as-4SU(20)	p*-UGC ^G GUAGCCUU
	EGFP-ss-5'OCH ₃	UUACGCGAGGACCUGCAUCGTm
l	EGFP-as-5'OCH ₃	mTGC ^G GUAGCCUU
	EGFP-ss-4SU(20)	UUACGCGAGGACCUGCAUCGG-p*

U denotes 4-thiouracil; mT denotes 5'-O-methyl thymidine; underline denotes nucleotide that is mismatched to its complementary sequence; italics denote DNA strands; p denotes 5'-phosphorylation; * denotes 5'-phosphorylation with $\gamma^{32}\text{P}$.

Table 6 – Statistical analysis (*p*-values) for data presented in Figures 4 and 5.

Asymmetric siRNA

	a	b	c	d	e	f	g	h	i	j
a										
b	<1.0E-04									
c	6.7E-01	<1.0E-04								
d	1.7E-04	<1.0E-04	<1.0E-04							
e	2.8E-01	<1.0E-04	5.1E-04	<1.0E-04						
f	3.0E-04	1.4E-01	3.0E-04	9.0E-04	2.0E-04					
g	4.0E-03	7.9E-03	6.3E-03	2.6E-02	5.6E-03	2.1E-03				
h	1.3E-03	4.9E-01	2.0E-03	2.8E-03	1.9E-03	4.7E-01	1.1E-02			
i	3.4E-01	<1.0E-04	2.7E-03	<1.0E-04	1.9E-01	2.0E-04	5.8E-03	2.0E-03		
j	1.6E-01	<1.0E-04	1.3E-01	<1.0E-04	3.4E-01	2.0E-04	4.9E-03	1.8E-03	2.8E-01	

Results are reported as *p*-values from unpaired, two-tailed Student's *t*-test assuming unequal variance performed using Microsoft Excel for each pair-wise comparison between siRNA "Fraction Crosslinked" data. Statistically significant differences (highlighted) are for $p < 5 \times 10^{-2}$. *p*-values less than 1.0×10^{-4} are indicated by $<1.0 \times 10^{-4}$. Note that E in the table means 10 raised to the power (e.g., $<1.0\text{E-}04$ means $<1.0 \times 10^{-4}$).

Symmetric siRNA

	a	b	c	d	e	f	g	h	i	j
a										
b	<1.0E-04									
c	9.2E-02	<1.0E-04								
d	<1.0E-04	1.9E-01	<1.0E-04							
e	3.6E-02	<1.0E-04	1.5E-01	<1.0E-04						
f	1.9E-03	4.1E-03	2.2E-03	3.7E-03	2.4E-03					
g	2.6E-03	4.9E-01	4.3E-04	4.4E-01	8.4E-04	3.7E-03				
h	4.0E-03	3.1E-01	9.9E-04	7.4E-01	1.6E-03	3.7E-03	6.7E-01			
i	4.5E-01	<1.0E-04	6.8E-02	<1.0E-04	2.9E-02	1.9E-03	2.0E-03	3.3E-03		
j	1.4E-01	<1.0E-04	1.5E-01	<1.0E-04	5.0E-02	1.9E-03	3.6E-03	5.2E-03	2.1E-01	

Results are reported as *p*-values from unpaired, two-tailed Student's *t*-test assuming unequal variance performed using Microsoft Excel for each pair-wise comparison between siRNA "Fraction Crosslinked" data. Statistically significant differences (highlighted) are for $p < 5 \times 10^{-2}$. *p*-values less than 1.0×10^{-4} are indicated by $<1.0 \times 10^{-4}$. Note that E in the table means 10 raised to the power (e.g., $<1.0\text{E-}04$ means $<1.0 \times 10^{-4}$).

EGFP siRNA

	a	b	c	d	e	f	g	h	k	l
a										
b	<1.0E-04									
c	1.5E-01	<1.0E-04								
d	1.1E-04	<1.0E-04	3.0E-03							
e	5.9E-01	<1.0E-04	2.2E-01	<1.0E-04						
f	<1.0E-04	<1.0E-04	<1.0E-04	<1.0E-04	<1.0E-04					
g	<1.0E-04	2.8E-01	<1.0E-04	1.9E-03	<1.0E-04	<1.0E-04				
h	<1.0E-04	9.0E-01	<1.0E-04	<1.0E-04	<1.0E-04	<1.0E-04	3.2E-01			
k	4.8E-04	5.2E-02	6.3E-04	5.1E-03	6.8E-04	2.8E-02	2.6E-02	4.9E-02		
l	4.2E-04	3.9E-01	6.5E-04	1.2E-02	6.9E-04	9.0E-04	1.8E-01	3.6E-01	1.9E-01	

Results are reported as *p*-values from unpaired, two-tailed Student's *t*-test assuming unequal variance performed using Microsoft Excel for each pair-wise comparison between siRNA "Fraction Crosslinked" data. Statistically significant differences (highlighted) are for $p < 5 \times 10^{-2}$. *P*-values less than 1.0×10^{-4} are indicated by $<1.0 \times 10^{-04}$. Note that E in the table means 10 raised to the power (e.g., $<1.0\text{E-}04$ means $<1.0 \times 10^{-4}$).